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National Status and Trends Program for Marine Environmental Quality Benthic Surveillance Project: Cycle III Field Manual

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Rockville, Maryland
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NOTICE

CONTENTS

LIST OF FIGURES

LIST OF TABLES

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THE NATIONAL STATUS AND TRENDS PROGRAM FOR MARINE ENVIRONMENTAL QUALITY BENTHIC SURVEILLANCE PROJECT: CYCLE III FIELD MANUAL

Gunnar G. Lauenstein
David R. Young

1. INTRODUCTION

The Benthic Surveillance Project (BSP) is a major component of NOAA's National Status and Trends (NS&T) Program. It is a collaborative effort between the Ocean Assessments Division (OAD) of the National Ocean Service and the National Marine Fisheries Service (NMFS).

The major goals of the Project are to describe present levels of chemical contamination in surficial sediments and bottom-feeding fishes at key sites in the nation's estuaries and nearshore zone, and to determine the incidence of disease in these benthic species. By repeating the survey yearly, trends in contamination and disease levels are being sought in order to determine whether the environmental quality of the target sites is improving or degrading. The Project was begun in 1984, and the third annual survey (Cycle III) is now in progress.

Because this is a national program conducted by scientific teams from various laboratories around the country, it is important that sample collection and processing procedures be standardized as much as possible. This manual is based both on the field experience gained by NMFS personnel during Cycles I and II, and the general expertise of OAD and NMFS scientists in conducting environmental quality surveys. The protocols in this manual will be followed by all Benthic Surveillance Project participants. As data from the Project are evaluated, the procedures, specified herein, will be modified and improved.

2. GENERAL FIELD SAMPLING PROCEDURES

Certain aspects of field sampling that are common to the collection of both fish and sediment samples are summarized here and described in more detail in the following sections:

A. The following information regarding the collection and processing of samples is required by the National Status and Trends Program. Essential information shall include: site (name and number, and latitude and longitude to tenths of a minute), sampling date, name(s) of person(s) collecting samples, type(s) of samples, method of storage aboard vessel, date of transport from vessel to storage facility, means of transport (e.g. frozen, packed in dry ice), arrival date, person in charge of transport, person receiving samples, and method of final storage.

B. Field operations (cores and trawls) may be done in any order, fish may be caught anywhere within a site, all the required fish may be caught in one tow, and positions of sediment stations are to approximate sediment stations occupied during Cycle I and II as closely as is practicable. Positions of sediment stations may not be coincident with the locale where fish are obtained, but must be in the general vicinity of the trawl track(s).

C. Geographic coordinates, or LORAN rates, and water depth shall be recorded for all "over the side" vessel operations. At sediment stations a position fix shall be recorded for each grab. For fish collections, the starting position and either the ending position, or the compass heading, speed and duration of trawl for each trawl shall be recorded. The sediment collection stations and trawl transects shall be plotted on a chart of appropriate scale, and photocopies shall be included in the cruise report.

D. All samples for chemical analysis shall be frozen in the field at -20°C, with the exception of those samples collected for the National Status and Trends Specimen Bank. Samples archived by the NMFS shall be stored at -80°C upon return to the laboratory.

E. Fish specimens for dissection shall be processed in a positive pressure clean air work station. The station is to be equipped with both a washable prefilter and a High Efficiency Particulate

Air (HEPA) filter with non-metal separators, characterized by a particulate removal efficiency of at least 99.7% for particulates larger than 0.3 microns. After every two weeks of use, the prefilter shall be replaced with a clean unit, and the used prefilter then shall be sprayed with a stream of fresh water and air dried in the laboratory before being stored in a plastic bag for reuse. Sediment samples, although exposed to the atmosphere during collection, are assumed to contain natural levels of analytes sufficiently high to preclude analytical artifacts resulting from this exposure.

F. Implements and containers used for processing and storing specimens and samples shall be fabricated only from certain allowable materials. Samples for metals analyses shall be handled and stored using only materials fabricated from plastic, except that dissections shall be performed using stainless steel and titanium implements as described in a following section. Such samples for metals analyses (fish tissue and sediment cores) shall be stored in the field in plastic containers and clear plastic (e.g., butyrate, polyethylene, polypropylene) core tubes, respectively. These containers shall have been prepared in the laboratory prior to use by rinsing with dilute (5-10%) nitric acid (HNO_3) or hydrochloric acid (HCl), followed by a deionized water (DI H_2O) rinse, and air drying.

Samples for organic chemical analyses (fish tissues and sediments) shall be handled using stainless steel and titanium implements, and shall be stored in glass jars sealed with Teflon lined caps. Stainless steel implements, glass jars and Teflon liners shall have been prepared in the laboratory prior to use by rinsing with spectral grade methylene chloride (CH_2Cl_2) and air drying. Stainless steel, titanium, glass, and Teflon materials, prepared as above, are the only materials suitable for contact with samples for organic analyses during collection or subsequent handling.

G. High purity reagents shall be used for all procedures. Organic reagents shall be of a purity to be specified by NOAA's National Analytical Facility. Mineral acids (e.g., HNO_3, HF, HCl, and H_2SO_4) shall be Ultrex, Suprapur or G.F. Smith (double distilled from Vycor glass or equivalent). DI H_2O shall be of 18 megohm-cm resistivity.

H. During Cycle III, one fish species shall supply tissue for chemical and histological analyses. At a given site, an effort shall be made to capture the same species obtained during Cycles I and II. If different species were sampled for chemical analysis during Cycles I and II, and both are available in sufficient quantities in Cycle III, that species collected at the greatest number of sites shall be selected as the target species.

I. Tissues sampled for histopathological examination shall be stored outside the clean air work station in Dietrich's fixative or 10% neutral buffered formalin. All three tissues (liver, kidney and gill) from each specimen may be stored in a single jar.

J. A color photographic record of all unusual fish lesions or conditions shall be made.

3. COLLECTION OF FISH

Fish may be collected by a series of alternative methods depending on field exigencies. The primary sampling tool shall be an untreated nylon bottom trawl net. An untreated seine net also may be used, depending on the location being sampled. Short trawls (approximately 15-20 minutes) shall be made to minimize net damage to captured specimens. An effort shall be made to obtain sufficient numbers of the target species of a single size class of fish prevalent at a given site. If sufficient numbers are not available, the following options are provided to improve the likelihood of obtaining adequate numbers of samples. These options will be

exercised in the following order at the discretion of the field party chief:

a. Relocate the site at some minimal distance from the original site.

b. Take specimens from two or more size classes.

c. Change from the target species to the alternate species that is most consistent with the species collected previously (Cycle I and II) in the region by the Benthic Surveillance Project, and is available in sufficient numbers for chemistry and histological analysis (see Table 1).

Table 1
Cycle I and II Species Collection List

Site	1984 Species	1985 Species
Casco Bay	Winter flounder	Longhorn sculpin
Penobscot Bay		Longhorn sculpin
Machias Bay		Longhorn sculpin
Frenchmans Bay		Longhorn sculpin
Merrimack R.	Winter flounder	Winter flounder
Salem Harbor	Winter flounder	Winter flounder
Boston Harbor	Winter flounder	Winter flounder
Buzzards Bay	Winter flounder	Winter flounder
Narragansett Bay	Winter flounder	Winter flounder
Long Island Sound East	Winter flounder	Winter flounder
Long Island Sound West	Winter flounder	Winter flounder
Raritan Bay		Winter flounder
Great Bay		Winter flounder
Delaware Bay Upper		
Delaware Bay Middle	Windowpane	Windowpane

Chesapeake Bay North	Atlantic croaker	Spot
Chesapeake Bay South	Atlantic croaker	Spot
Pamlico Sound	Atlantic croaker	Atlantic croaker
Charleston Harbor	Atlantic croaker	Atlantic croaker
Sapelo Sound	Spot	Atlantic croaker
St. Johns River	Spot	Atlantic croaker
Charlotte Harbor	Spot	Spot
Tampa Bay		Sea catfish (histology only)
Apalachicola Bay	Atlantic croaker	Atlantic croaker
Pensacola Bay		Atlantic croaker
Mobile Bay	Atlantic croaker	Atlantic croaker
Round Island	Spot	Atlantic croaker
Heron Bay	Atlantic croaker	Atlantic croaker
Miss. River Delta	Atlantic croaker	Atlantic croaker
Barataria Bay	Atlantic croaker	Atlantic croaker
Galveston Bay	Atlantic croaker	Atlantic croaker
San Antonio Bay	Atlantic croaker	Atlantic croaker
Corpus Christi Bay	Atlantic croaker	Atlantic croaker
Lower Laguna Madre	Atlantic croaker	Atlantic croaker
San Diego Harbor	Barred sand bass	Barred sand bass
	Diamond turbot	
San Diego Bay		Spotted sand bass
	Spotted turbot	Spotted turbot
	Hornyhead turbot	Hornyhead turbot
Dana Point	Hornyhead turbot	Hornyhead turbot
	White croaker	White croaker
	Barred sand bass	Barred sand bass
San Pedro Bay (outside)	Hornyhead turbot	
		White croaker
Seal Beach	Hornyhead turbot	
	White croaker	
Long Beach		White croaker
Santa Monica	Hornyhead turbot	Hornyhead turbot
Morro Bay		
Monterey Bay		English sole
San Francisco Bay		
Oakland	White croaker	White croaker
Hunters Point	Starry flounder	
Southhampton Shoal	Starry flounder	Starry flounder
San Pablo Bay	Starry flounder	Starry flounder
Bodega Bay	White croaker	White croaker
	Starry flounder	Starry flounder
		English sole
Humboldt Bay		
Coos Bay	Starry flounder	Starry flounder
Columbia River	Starry flounder	Starry flounder
Elliott Bay	English sole	English sole

Nisqually Reach	English sole Staghorn sculpin	English sole
Commencement Bay	English sole	English sole
Nahku Bay	Flathead sole Yellowfin sole	
Lutak Inlet	Flathead sole	
Prudoe Bay		
Simpson Lagoon		Fourhorn sculpin
Endicott		Fourhorn sculpin

A set of at least 30 individual tissue samples is required for each of three types of analysis: histopathological, organic chemical* and trace metal/archive. As target specimens are landed aboard, the length of each individual shall be measured and recorded on a length-frequency histogram, and the specimens placed in a tank of running seawater (seawater with a bubbler may also be used) or, as a last resort, packed on ice in a chest with an open drain so that water does not accumulate and immerse the specimens. As the histogram develops, the prevalent size class to be sampled will emerge.

At Northeast and West Coast sites, where separate portions of individual livers must be used for histopathology, organic chemistry, and trace metals, the following steps shall be used:

A. Select an individual specimen of the prevalent size class and assign it a unique specimen number.

B. Measure and record the specimen's total length (to the nearest mm) and weight (to the nearest gram).

C. Perform an external examination, and record any anomalous condition (e.g., external tumor, eroded fins).

* 60 individuals are required for the southeast/Gulf sites.

D. Using a filleting knife or a scalpel (but not a titanium knife), sacrifice the specimen by severing the spinal cord. Remove the second gill arch and preserve it for histopathology (except for specimen bank samples). Wipe the body with a low-lint paper or cloth towel to remove as much mucus as is practical.

E. Transfer the fish specimen onto a lint-free cotton cloth in the clean air work station. (Note: Only lint-free cotton cloths are to be used in the work station.)

F. One set of dissection tools (comprised of high-quality stainless steel scalpel, scissors, hemostat, forceps, and custom-made knifes with titanium blade and Teflon handle) will be provided for each clean air work station, with four groups of tools per set as follows: Group 1 - scissors and forceps; Group 2 - sharp-pointed scalpel, scissors, forceps or hemostat, and Ti knife; Group 3 - scalpel or scissors, forceps or hemostat; Group 4 - forceps and Ti knife (The addition of a Ti or Teflon scoop to Groups 2 and 4 also is anticipated).

Before initiating a set of dissections, and after sharpening the Ti knife if necessary, the tools should be wiped clean of extraneous material, scrubbed in detergent solution, rinsed extensively with tap water, rinsed in DI H_2O, transferred to the fume exhaust hood where they will be carefully rinsed with CH_2Cl_2, and carried on a similarly cleaned Teflon cutting board to the work station. There they are transferred to clean lint-free cotton cloths at the rear of the work area.

Following a given step in the dissection process, and between specimens of a dissection set, the tools are cleaned by wiping off adhering tissues using a lint-free cotton cloth, rinsing with DI H_2O, and shaking dry. Gloves made of vinyl or other synthetic material (even if talc- or dust-free) shall not be worn during the dissection process. Instead, the hands should be thoroughly washed using a bar of Ivory soap.

G. Using the scissors or scalpel and forceps from the first tool group, open the body cavity and record the gender and, if possible, the state of reproductive development (except for Specimen Bank specimens). Wipe off the tools with a lint-free cloth, rinse them with DI H_2O, shake dry, and return them to their Group 1 position (cloth) within the work station.

H. Using the dissection tools from Group 2, separate the gall bladder from the liver, being sure to grip it by the bile duct to prevent bile from flowing out of the bladder. Hold the bladder at the mouth of the glass vial designated for this sample, and puncture the bladder with the scalpel blade. Allow the tip of the blade to touch the inside rim of the mouth of the vial, thus conducting the bile fluid (for PAH analysis) into the vial. Wipe adhering bile from the scalpel blade, rinse with DI H_2O, and shake dry. (Bile is not collected from Specimen Bank samples).

I. Using the same tools (from Group 2), free the liver from surrounding tissues. Place it on a clean Teflon cutting block, and using the Ti knife, longitudinally cut it into three pieces with the mid-section not more then 3 mm thick. Store mid-section in the vial of fixative (either Dietrich's or buffered formalin) containing the gill arch.

J. Using the same tools, remove one-half the remaining liver and store in a glass jar (for organic chemical analysis). Remove the other half and store in a plastic vial (for trace metal analysis). Every attempt should be made to avoid or minimize contacting the "trace metal" half of the liver with stainless steel tools.

K. Using the same tools, remove whole stomach or empty its contents and store in a glass jar (for organic chemical analysis).

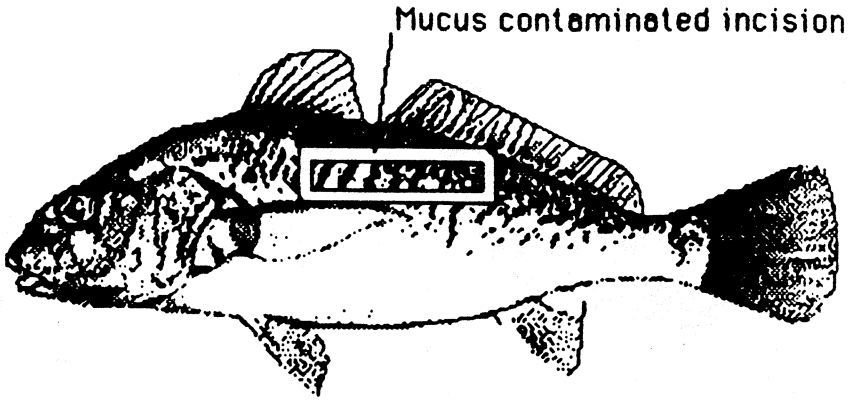
L. Using the same tools, remove the kidney and store in the vial of fixative with the gill arch and liver section. Then wipe off the tools with a lint-free cotton cloth, rinse with DI H_2O and shake dry.

M. Begin the dissection of the muscle tissue sample with the stainless steel scalpel or scissors, from the third group of tools. (If the specimen is a flatfish, place it with the pigmented side facing up.) A series of four cuts is made into the dorsal section to obtain a rectangular subsection of muscle (Figure 1). The first cut extends from behind the head almost to the tail, just above the lateral line. The second is parallel to the first, just below the fin ridge. The two final cuts are made perpendicular to these cuts to obtain the rectangular subsection. The scalpel is wiped, and rinsed with DI H_2O between cuts to remove scales and as much mucus as possible.

N. Use the scalpel from the third group to lift the edge of the skin along the cut line at the posterior end of the rectangular cut. Then hold the fish tail with one hand, and use the forceps or hemostat in the other hand to tightly grasp the free edge of the skin. Pull the skin back off the rectangular cut to expose the muscle tissue mass (Figure 2). (Note: A layer of adipose tissue lies along the dorsal fin ridge. This tissue is not to be taken with the muscle tissue subsample.)

O. Use the titanium knife (or scoop) from the fourth group to obtain a "core" of the muscle tissue mass within the rectangular cut (Figure 3). Extreme care must be taken to assure that neither the contaminated rectangular cut line (including the area where the skin originally was lifted) nor the fish exterior is contacted either by the Ti knife or by the cored muscle sample. The Ti knife (or scoop) is then used to transfer this uncontaminated muscle tissue core to a glass jar.

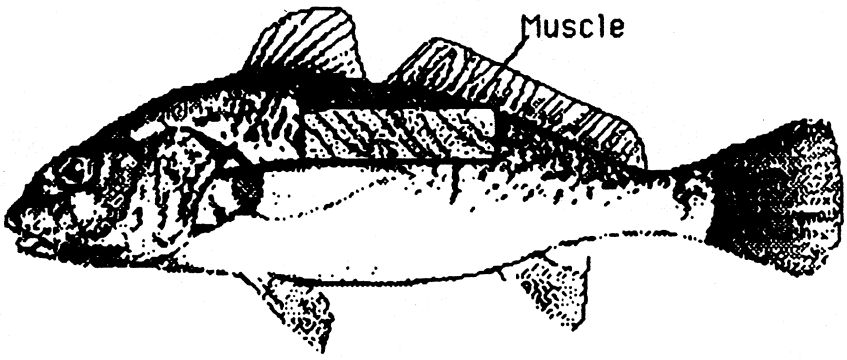
P. Remove the carcass from the work station. Use a filleting knife to cut off the head, and store in a plastic bag for subsequent removal of the otoliths. Discard the carcass.



ATLANTIC CROAKER (Micropogon undulatus)

Step M. Muscular incision using stainless steel knife or scissors.

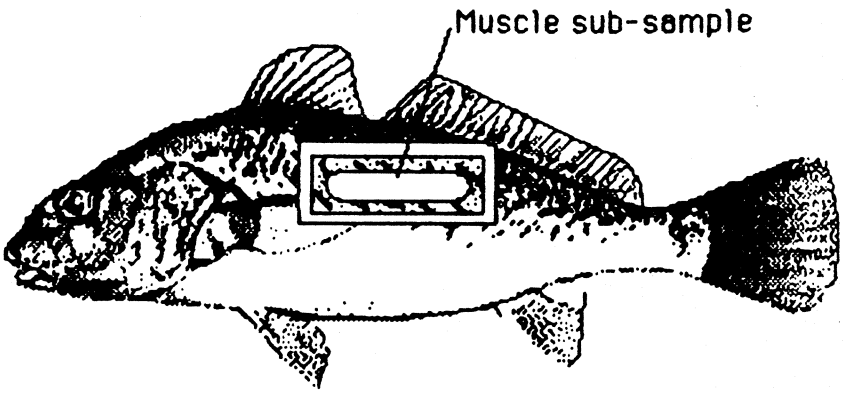
Figure 1. First step (of three) in fish muscle dissection.



ATLANTIC CROAKER (Micropogon undulatus)

Step N. Epidermis torn away exposing underlying muscle.

Figure 2. Second step (of three) in fish muscle dissection.



ATLANTIC CROAKER (Micropogon undulatus)

Step 0. Muscular sub-sample taken with titanium knife.

Figure 3. Third step (of three) in fish muscle dissection.

Q. Use DI H_2O water to clean dirty areas of the work station surface, wipe with a lint-free cloth, and repeat the process for the next specimen of the dissection set. Tools are to undergo a complete cleaning after each site's dissections are completed.

At sites in the Southeast/Gulf region where fish livers are not of sufficient size for combined use, a minimum of 120 fish livers shall be collected in the field. The histology set shall include 30 specimens processed as described above but substituting whole livers. The organic chemistry set shall include 60 specimens processed as described above but substituting whole livers. The trace metals set of 30 specimens shall be frozen individually in plastic bags and dissected in the laboratory following the general procedure described above but substituting whole livers.

The steps listed above are summarized in the flow diagram presented in Figure 4.

4. PROCEDURE FOR COLLECTING FISH TISSUE SAMPLES FOR THE NATIONAL STATUS AND TRENDS SPECIMEN BANK

A. The amount of each sample to be banked shall be approximately 150g. Since it is desirable to bank true replicates (aliquots A and B) of each sample, approximately 300g (2x150g) of each tissue (muscle and liver) shall be collected at each site.

B. The number of individual fish to be pooled will be determined by the average size of the fish liver. Ideally, equal amounts of liver and muscle tissue should be collected from each fish. If the amount of obtainable liver is limited due to size and/or number of fish at a specific site, the ratio of liver to muscle can be adjusted to 2:3 to obtain a <u>full 2x150g</u> muscle tissue sample and a minimum 2x100g liver sample.

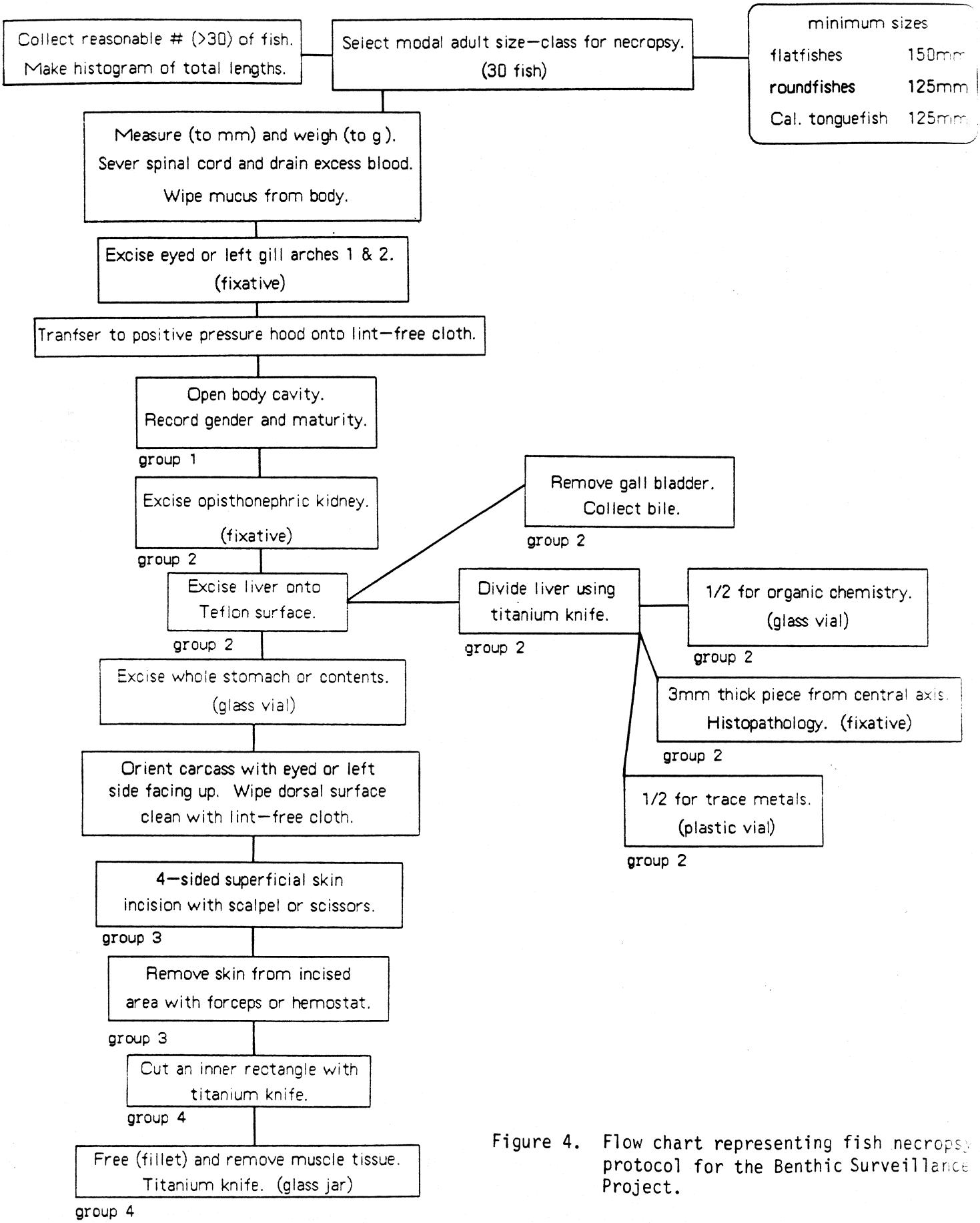


Figure 4. Flow chart representing fish necropsy protocol for the Benthic Surveillance Project.

C. Measure and record the specimen's total length, and plot (on the sample record form provided) to obtain a histogram. Fish selected for the Specimen Bank Samples should approximate the size distribution characteristic of the Benthic Surveillance samples.

D. Begin the dissection in the same manner as outlined in steps D-F (page 8). Using the scissors from the first tool group, open the body cavity, and record the gender. The total numbers of males and females collected should be recorded on the supplied sampling form.

E. Using the tools from the second group, free the liver from the surrounding tissue. Place the liver onto a cleaned Teflon block, and, using the Ti knife, divide it into equal parts for the A and B sample. Wipe the implements with a lint-free cloth rinsed with DI H_2O after each dissection.

F. To obtain fish muscle, follow steps M-O outlined on page 10. Muscle tissue is divided into A and B replicates for each fish. The A and B portions are placed on the provided Teflon blocks, and should approximate the weight of the liver sample to be taken.

G. The A and B parts of each tissue should be placed into two separate, lint-free Teflon bags supplied by NBS. Teflon bags and sheets are packaged under clean room conditions in larger Teflon bags. These materials should only be opened in the clean air work station; the remaining bags shall be resealed in the original Teflon bag.

H. Because a partial tissue sample (i.e. less than the required 150g) must be stored for short intervals to obtain the total sample, the Teflon bag should be placed in a covered glass jar and cooled on ice or, for longer periods, refrigerated. After collection of the complete pooled sample, proceed with packing and shipment procedures.

Interim storage of tissue and sediment shall not exceed one day. Sediment collection does not extend beyond one day but fish collection occasionally does; in this case tissue samples shall be packaged in Teflon bags and frozen in liquid nitrogen (LN_2) at the end of the sampling day. Specimen Bank tissues collected during a second sampling day shall be packaged and frozen (LN_2) in a second set of Teflon bags.

The steps listed above are summarized in the flow diagram presented in Figure 5.

5. COLLECTION OF SEDIMENT

Surface sediments shall be collected using a box corer. A Smith-MacIntyre or modified Van veen grab sampler may be used as a backup sampling device. Three box cores shall be obtained at each station, totaling nine box cores per site. Four separate samples shall be taken from each box core, one surface skim (top 3 cm), and three cores. Each sample collected shall be given a unique specimen number. Before the first deployment of the box corer at each station, the sample compartment will be rinsed with seawater, then with CH_2Cl_2. Before subsequent deployments at the same station, the sample compartment need only be rinsed with seawater.

After retrieval of the box corer, the sample compartment shall be opened and its contents visually inspected to insure that the desired substrate (fine, depositional material) is present and that the sediment surface is undisturbed. A bed of sediment with 2-5 cm of overlying water will be considered optimal. Intact presence of worm tubes and other delicate structures indicates the sediment surface was not disturbed during sampling. If unacceptable, the sample shall be discarded and another obtained.

Flocculent material may be present in the overlying water, depending upon the location. Before draining the water, the plastic core tubes will be inserted into the sediment, capturing a shallow column of water in the core over the sediment surface. Flocculent material, if present, shall then be included as part of the sample.

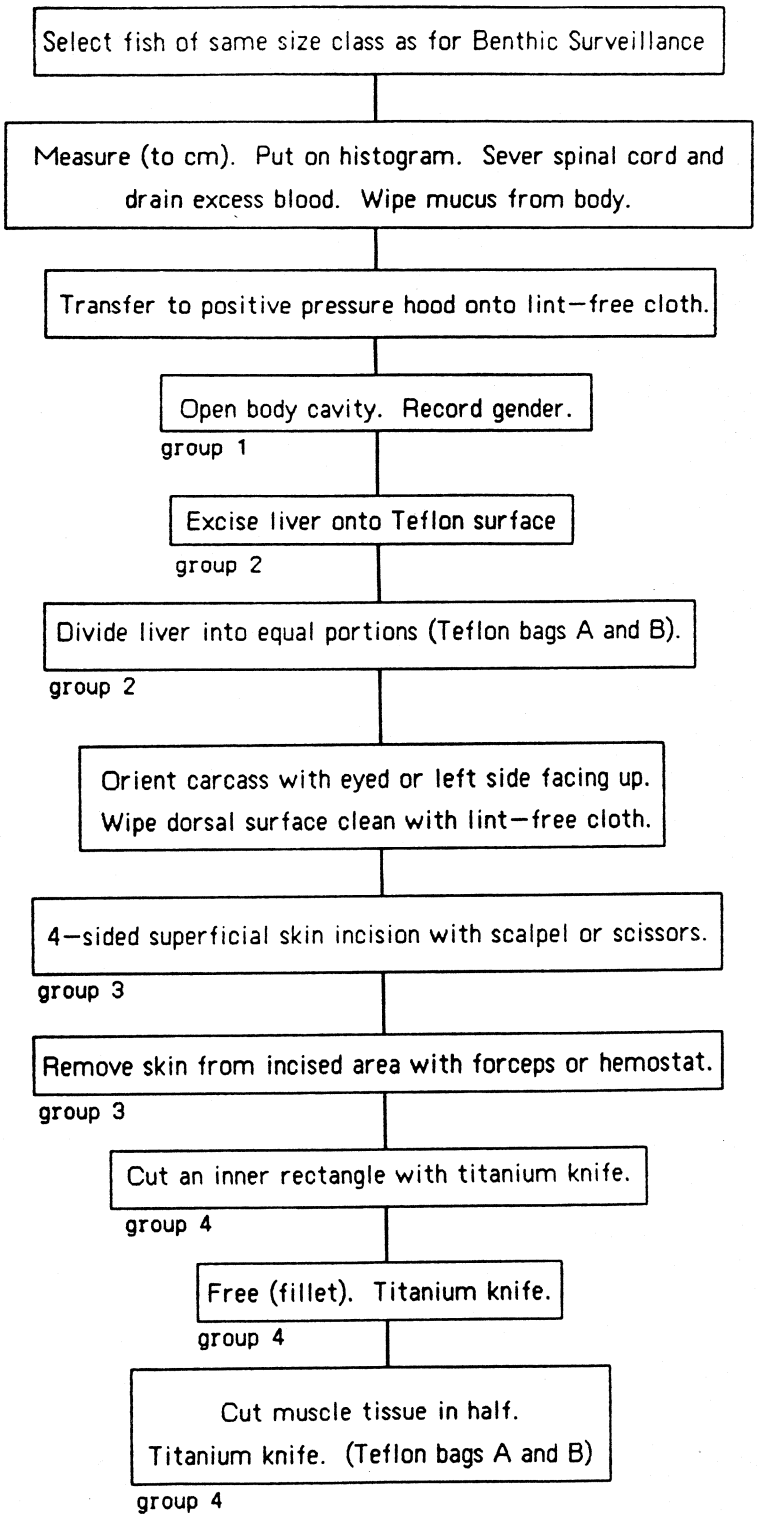


Figure 5. Flow chart representing fish necropsy protocol for the Specimen Bank Project.

The sub-samples should be taken from that area of the sediment sample that appears to be least disturbed, but away form the sides of the sampler to avoid contamination.

After the plastic core tubes have been inserted into the sediment, they shall be capped and the remaining overlying water shall be drained slowly, to prevent disturbance of the remainder of the sediment surface. When all the water has been drained, the center portion (10 cm L x 10 cm W x 3 cm D) of the sediment surface (for organic chemical analysis) shall be removed using a stainless steel scoop, freshly rinsed with CH_2Cl_2, and stored in a glass jar. Cores (for analysis of trace metals, grain size and TOC, and for archiving) shall then be removed, capped, and stored frozen upright.

6. PROCEDURE FOR COLLECTING SEDIMENT SAMPLES FOR THE NATIONAL STATUS AND TRENDS SPECIMEN BANK

A. A sediment sample is required from each selected site for specimen banking. The sample shall consist of pooled cores from three stations per site.

B. Of the three box cores or grabs to be taken at each station for the Benthic Surveillance Project, one also shall be used for the Specimen Bank Project. From one of the box cores at each station, two equivalent cylindrical core samples of approximately 40mL shall be taken; one shall be designated as portion A and the other as portion B. The A portions from each station will be pooled to provide the sample A (120mL) for the site; the portions B will provide the sample B respectively.

C. The cylindrical cores shall be taken with the supplied Teflon tubes. A core length of 30mm equals 40mL of sediment. The samples are taken by inserting the cylinders into the box core, closing the top with the screw cap, and withdrawing the core from

the box core. The bottom end is also capped for intermediate storage. The cores shall be stored on ice or in the freezer until all six have been collected.

D. The A and B portions of the sediment core shall be extruded under the laminar flow hood into two separate, lint-free Teflon bags.

E. The samples are extruded with the aid of the foam plugs which are inserted into the bottom of the tubes. When a seal is achieved, the top cap can be unscrewed. The water on top is now removed with a pipette (pasteur), or the corer containing the sediment may be held over a beaker allowing the contained water to overflow into the beaker as the sediment is pushed upwards. If not already the case, the sediment is pushed upwards until the surface reaches the rim of the tube. Use one of the 30 mm long labels to mark the length of the path over which the plug must be pushed upwards. Push the plug to the marked endpoint and a 30 mm sediment core will be above the rim of the tube. Use a titanium knife to cut off this portion and place it into the appropriate Teflon bag. (If the sediment core is too fluid to permit an accurate extrusion and collection of the sediment plug, the core tubes may be placed in a freezer until just frozen, and then extruded).

F. After collection of the complete pooled sample, proceed with packing and shipment procedures.

7. PACKING AND SHIPMENT OF NATIONAL STATUS AND TRENDS SPECIMEN BANK SAMPLES

A. When placing the tissues or sediments in the bags, care should be taken to avoid getting fluid on the Teflon surface to be sealed. It is particularly difficult to get a good seal on wet Teflon surfaces. If moisture should get on the rim of the Teflon bag it

may be removed using a clean lint free cotton cloth, or the partially sealed bag may itself be bagged. After collection of the complete pooled sample, the Teflon bags containing the tissue or sediment samples are then heat sealed. Workable settings on the heat sealer are marked (1.1 seconds for the impulse time, and 4 seconds for the cooling timer). However, if sealing problems still occur, vary the current/temperature and duration settings.

B. It is important to seal the bag with as little air remaining inside as possible. The air may be squeezed out or vacuum applied to a partially sealed bag. Alternatively, seal the bag with as little air remaining as possible; then squeeze the remaining bubble of air to a corner, puncture this corner with the titanium knife, squeeze out the remaining air and reseal the corner. The seal can be tested by gently squeezing the bag to see if it holds pressure. A second seal is to be made slightly away from the first and parallel to it to provide a double seal.

C. The pooled samples are weighed using an empty bag to tare the weight of the Teflon bag. The weights are recorded on the sampling data form and the sample labels. The labels are affixed to sample bags.

D. Each sample is then placed into a second bag together with the label and sealed again (as in step B above).

E. The double-bagged samples are placed in the cylindrical cardboard containers. The cardboard cylinders are then labelled with sample type, weight in grams, location and date of collection, and cruise number. The cardboard cylinders are then immersed in liquid nitrogen (LN_2) for 10 minutes to freeze them. The LN_2 shipper should not be used for freezing the samples, but only for shipping. If no separate supply of LN_2 is available, it may be convenient to fill the shippers completely with LN_2 before departure on sampling trips. Excess LN_2 may be used to quick-freeze the samples in the supplied dewars.

F. The LN_2 shipper should be filled with liquid nitrogen for at least six hours to fully prepare it for shipping. This time is necessary to fully saturate the absorbent inside the shipper. Before placing the frozen samples in the shipper, the excess LN_2 must be poured off.

G. The bagged, boxed, and frozen tissue sections are then transferred to the LN_2 shipper for storage. The large shippers can hold up to 10 sample boxes. Once the shippers are full they are to be shipped to the National Bureau of Standards; the samples are not to be stored in intermediate freezers.

H. Make sure that all entries in the Specimen Bank Sample Data Form (attachment) are complete. Any deviations or modifications of this protocol must be noted on the sampling form. In addition to the forms provided, include a copy of the applicable site log containing station latitude and longitude, geographic site name and associated National Status and Trend's assigned site number. Place a copy of the completed forms in the shipper and retain another copy for the project records.

I. The frozen specimens and their corresponding sample record sheets should be shipped by an overnight express carrier. The shippers must not contain LN_2 when shipped. Maximum holding time for the large shippers is 10 days; for the small shippers, the holding time is 6 days. Send the shippers C.O.D. to National Bureau of Standards using the shipping labels provided; e.g. the Federal Express form has a box to check if the recipient is being billed. No account number should be supplied.

J. Please notify Specimen Bank personnel by telephone as soon as possible after the specimens are shipped: Barbara Koster (301) 921-2613, or if she is unavailable, Rolf Zeisler (301) 921-2166, or Steve Wise/Michele Schantz (301) 921-2154.

8. HANDLING OF LIQUID NITROGEN

Liquid nitrogen should not be stored in sealed containers. Personnel handling LN_2 are cautioned to wear boots, cuffless trousers, non-absorbent aprons, loose insulating gloves, and face shields.

NATIONAL BIOMONITORING SPECIMEN BANK

Sampling Data — NOAA NS&T Program

Benthic Surveillance Project

Sample Source

Site ID (geographic name) Lat. Long.

Sample Type: Liver Muscle Sediment

Time of collection: Liver/Muscle (day/mo/yr) (hr.) Sediment (day/mo/yr) (hr.)

Intermediate(temp/remark) Storage: _____

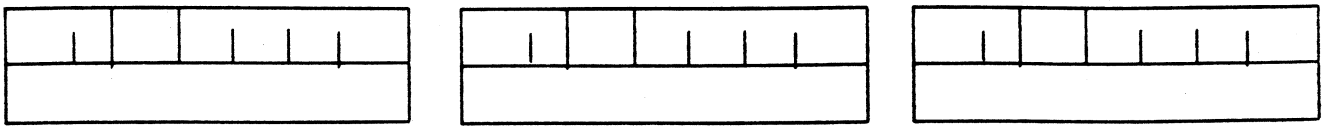
_____

Time of preparation: Liver/Muscle (day/mo/yr) (hr.) Sediment (day/mo/yr) (hr.)

Intermediate(temp/remark) Storage: _____

_____

Time of LN_2 freezing: (day/mo/yr) (hr.)



Fish species _____

Number & sex in sample A: _____ B: _____

Sediment grabs (log number) A: _____ B: _____

Sediment sample weight A [ ] g sample B [ ] g

Liver sample weight A [ ] g sample B [ ] g

Muscle sample weight A [ ] g sample B [ ] g

Protocol: Standard [ ] Modified [ ] Please note modifications below:

Remarks:

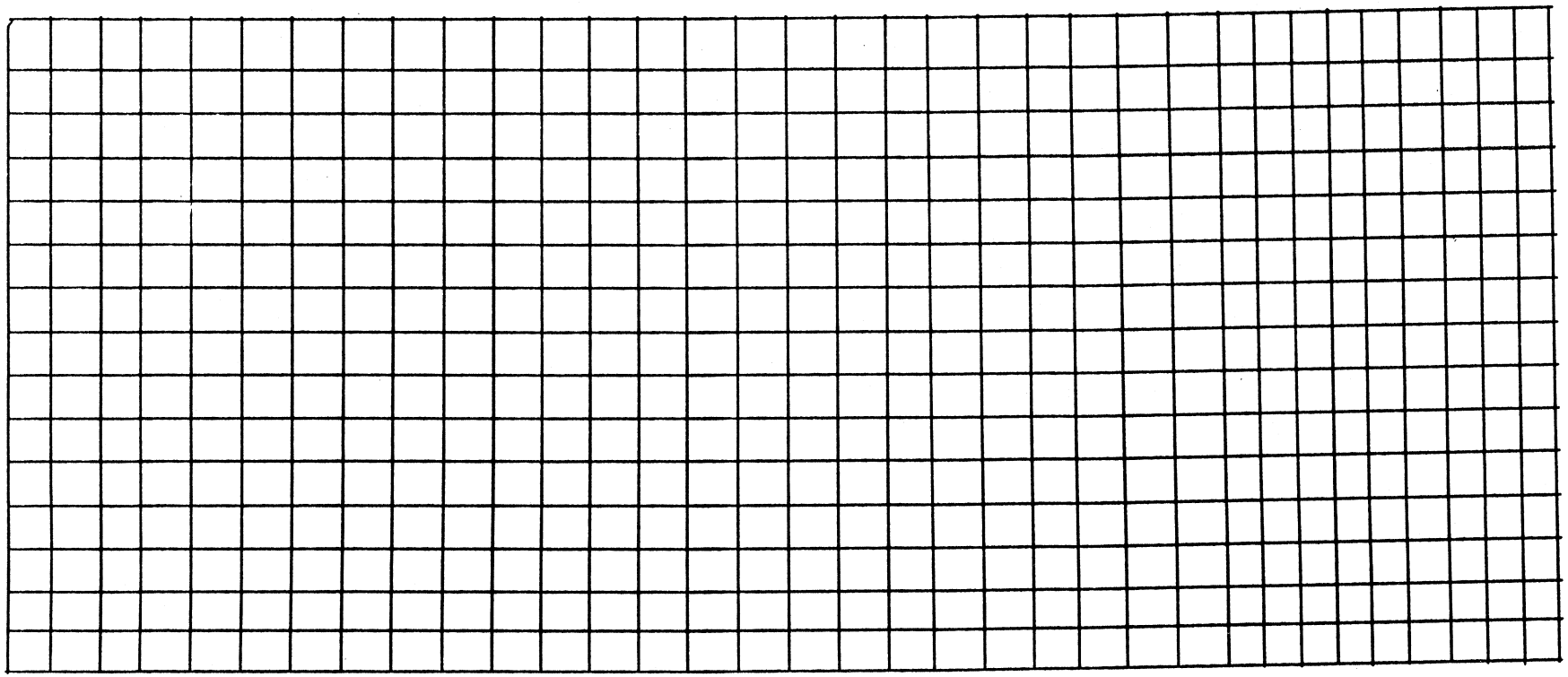
Prepared by: _____

Name (print)

_____

Signature

Fish Length Histogram



26

X axis to be labeled with appropriate size range in centimeters